PHOTOGRAPHIC ANALYSIS OF LIP FILLERS WITH HYALURONIC ACID

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ABSTRACT

This study aimed to evaluate volumetric lip changes with hyaluronic acid (HA) by comparing pixel values between preand post-filling lip photographs. Thirty-two female patients, aged 35 to 66 years, were selected and received 1 mL of HA, which was applied to both the lower and upper lips. In the before-and-after photographs, the changes in pixel count resulting from the HA injection were quantified using GIMP, a reliable, widely used image analysis tool. Paired t-tests and ANOVA were applied. Our study revealed significant increases in the left upper lip ($p = 2.73 \times 10^{-7}$), right upper lip ($p = 6.04 \times 10^{-6}$), lower lip ($p = 9.65 \times 10^{-5}$), and total lip area (p = 0.0019). Younger patients showed greater volumetric gain. Our findings demonstrate that lip filling with 1 mL of HA is effective and measurable, as evidenced by photographs of patients' lips. Additionally, pixel analysis is a reliable clinical tool that provides valuable insights into the effectiveness of lip augmentation, supporting the validity of our results.

Key words: Lip filling, Hyaluronic acid, Lip, Esthetics.

Introduction

Facial aging is a continuous, progressive process of loss of three-dimensional volume involving soft tissues, muscles, and bone structure [1]. The lips also suffer from the effects of aging, resulting in a loss of fullness and definition, which alters their anatomical shape and the desired proportions of the beauty relationship [2, 3]. The cutaneous portion of the upper lip increases, reducing the visualization of the upper incisors, and the nasolabial and labiomental grooves deepen due to the ptosis of underlying fat [4, 5]. The loss of muscle volume in the upper lip causes the philtrum and Cupid's bow to flatten and favors lip inversion into the oral cavity [4]. Clinically, there is also a loss of volume in the vermilion of the lips, which makes the lips thinner and less defined, in addition to ptosis of the labial commissure [4, 5].

Lip fillers have gained popularity because they create smooth lips by restoring shape, volume, contour, projection, and eversion, consequently enhancing beauty and youth [3, 6, 7]. They are the second most performed minimally invasive cosmetic procedures between 2020-21 in the United States, representing approximately 1.8 million people [8]. Lip fillers commonly use hyaluronic acid (HA) gel [9, 10]. This non-surgical procedure is often preferred due to its immediate results, outpatient treatment, reversibility, and reproducibility [2, 11-14].

The effectiveness of lip fillers can be assessed through photographic analysis. This approach allows detailed visual documentation of changes in the lips before and after the filler, facilitating the study of the results and communication between the patient and the professional [7]. The effectiveness of lip fillers can be evaluated using scales and software, such as the Bellus 3D® facial scanning application for cell phones [15].

Although there are studies on the use of HA for lip-filling techniques, these focus solely on evaluating the procedure's effectiveness through subjective photographic analysis before and after [9]. The use of computational analysis [16] could support better planning of the amount of material to be injected, the HA insertion sites, and the form of deposition, as well as assess the effectiveness of lip filling using precise, reliable measurements (vertical and horizontal projections).

Thus, this study aimed to evaluate the efficacy of lip fillers with HA by measuring vertical and horizontal projection distances and counting the number of pixels in pre- and post-photographs. The null hypothesis tested in this study was that there is no difference in lip measurements between photos taken before and after lip filler with HA.

Materials and Methods

This study is observational and cross-sectional, examining patients treated in a Private Office.

Sample

A preliminary and randomized selection of 74 medical records of patients was performed to identify female patients

aged between 35 and 66 years who had undergone the application of 1 mL Restylane Kysse HA (Galderma) for lip volumization. The inclusion criteria were: 1) medical records containing photographs taken before/after the procedure, 2) images with the most remarkable similarity in focal length, 3) photographs using the lens/sensor/camera set, 4) similar lighting conditions, and 5) photographs in which the patients were looking directly at the camera and with their eyes open. In turn, the exclusion criteria consisted of medical records with 1) male patients, 2) patients under 35 years of age and over 66 years of age, 3) photographs in which patients blinked/closed their eyes, 4) syndromic patients, 5) patients who underwent previous surgery in the lip region, 6) patients with cleft lip and/or facial trauma [17-24]. After applying the exclusion and inclusion criteria, 32 individuals remained.

Clinical evaluation and procedure

All patients were treated with 1 mL of HA in a clinical setting at a private practice under local anesthesia, performed by the same operator. The HA filler used was Restylane Kysse®, chosen for its high viscosity and non-pyrogenic properties, which are beneficial for lip volumization. It is a transparent, colorless, sterile, high-viscosity, cross-linked HA aqueous gel of synthetic origin. All cases were planned based on the labiogram, a digital analysis of the lips. The lip-filling technique performed was the same for all cases.

Initially, the filling was performed through two holes in the upper lip, one at the apex of the right Cupid's bow and the other at the left. From these, a cannula was introduced into the premolar region, and retroinjection with HA was performed until the cannula was removed entirely through the holes. Subsequently, a hole was made in the contour of the center of the lip in the lower arch, and from there, the cannula was introduced up to the labial commissure, and from there, retroinjection was performed. Finally, four retroinjections with a needle were conducted in the region of the contour of the upper labial Cupid's bow to promote eversion of the upper lip (Figure 1). The amount of HA distribution in the lower and upper lips was determined individually.



Figure 1. Photo before and after the hyaluronic acid filling procedure

Collected variables

The following variables were collected in each before and after photograph: 1) height of the left upper lip (BLUE); 2) height of the right upper lip (RED); 3) height of the lower lip (midline) (GREEN); 4) total area of both lips (YELLOW); 5) distance between the centers of the eyes (standard for scale normalization) (WHITE) (Figure 2). The primary outcome of interest was the measurement of the increase in lip measurements after the application of 1 ml of HA.



Figure 2. Measures evaluated. Source: AI-generated image, https://thispersondoesnotexist.com/.

Equipment and software

In the present study, all dimensional analyses were performed in relative units (pixels) to maintain internal consistency within each photographic pair. The use of pixelbased measurements enables the quantitative assessment of proportional changes between anatomical landmarks, minimizing bias related to image scaling while preserving the validity of comparative analyses. The use of pixel-based measurements provides a reliable and objective approach for quantifying morphological changes. This method allows for precise detection of proportional variations between anatomical landmarks, independent of potential differences in photographic scale or resolution. By analyzing relative distances in pixels rather than absolute linear values, potential measurement biases are mitigated, ensuring internal consistency within each image pair. Moreover, this approach facilitates reproducibility and comparability across different studies, serving as a robust digital metric for assessing aesthetic outcomes in facial analysis.

All images were processed using GIMP software version 2.10.24, and the pixels were collected at a zoom of 2300%. All data were compiled in Excel spreadsheets (Redmond, Washington, USA) and are available in Microsoft Office.

Statistical analysis

Initially, descriptive analysis was performed by calculating

absolute and percentage frequencies for categorical variables and the mean and standard deviation (mean \pm SD) for numerical variables, with distances measured at each point mentioned for each image (individual).

The inferential analysis was performed using the Student's t-test to assess the association between pre- and post-lip-filling measurement variations [25-30]. A One-Way ANOVA statistical test was performed to confirm the analysis results for the repeated distances measured at each site. The margin of error used in the decision of the statistical tests was 5% (p < 0.05). The data were entered into an Excel spreadsheet (Redmond, Washington, USA), and the program used for statistical calculations was BioStat

(Instituto Mamirauá-Conservação na Amazônia, Brazil). The data were summarized using tables that included descriptive statistics (means and standard deviations) and inferential statistics (p-values).

Results and Discussion

There was variation in the four measurements: 1) height of the left upper lip; 2) height of the right upper lip; 3) height of the lower lip (midline), and 4) total area of both lips, before and after filling (**Table 1**).

Table 1. Changes in lip measurements before and after filling

	Upper lip height (left):	Upper lip height (right):	Lower lip height:	Total area:	
Patient (no.)	Variation (%)	Variation (%)	Variation (%)	Variation (%)	Age (years)
2	19,2	17,6	3,0	15,8	23
22	13,8	14,9	27,8	35,4	27
6	28,9	9,6	13,0	21,7	29
25	19,2	5,1	-1,4	9,6	30
18	24,1	7,2	12,6	41,3	32
28	41,5	41,7	13,4	68,9	32
19	29,8	24,3	-14,0	-0,7	33
5	19,8	4,6	6,1	-1,5	34
17	48,8	56,7	9,1	9,7	34
21	13,6	22,4	8,0	5,4	34
29	18,6	21,0	22,7	42,0	34
4	7,79	4,3	17,5	33,1	38
26	10,2	1,1	9,6	24,9	38
32	44,4	54,8	208,8	102,1	38
16	22,8	29,1	20,9	29,3	41
3	28,9	50,4	37,5	31,6	42
9	11,8	14,8	9,9	12,3	44
13	17,6	9,2	36,5	41,9	44
15	0,8	0,8	38,6	58,7	44
1	10,4	7,5	8,5	14,1	45
14	10,5	10,5	18,6	18,0	45
31	10,5	14,6	17,0	27,2	45
8	26,0	22,4	62,5	59,6	46
23	36,4	40,8	37,2	58,2	46
7	17,1	11,0	-1,5	28,4	48
30	4,0	9,8	94,5	26,4	49
12	22,8	19,6	47,4	30,1	64
20	11,2	2,4	25,2	13,1	67
27	-5,9	-5,9	163,9	50,2	67
24	36,9	35,2	42,3	32,0	68

10	54,3	203,3	4,3	26,0	69
11	42,2	54,9	60,9	70,8	72

For each of these four parameters, paired t-tests were conducted to compare pre- and post-procedure values. Statistically significant differences were observed in all cases, indicating a consistent volumetric increase following hyaluronic acid (HA) application: left upper lip height (p = 2.73×10^{-7}), right upper lip height (p = 6.04×10^{-6}), lower

lip height – midline (p = 9.65×10^{-5}), and total lip area (p = 0.0019) (**Table 2**). These findings suggest that HA lip filling effectively enhances both vertical lip dimensions and total surface area, reflecting clinically perceptible improvements in lip volume and contour definition [31-36].

Table 2. Student's t-tests results for comparison between variations, before and after the examination, at each measured location.

Paired two-sample t-test for means Upper lip height (Left)			Paired two-sample t-test for means Lower lip height (midline)			
Average	38.53125	46.71204	Average	45,875	57.89669	
Variance	362,128	565,6263	Variance	560,8871	820.9888	
Observations	32	32	Observations	32	32	
Pearson Correlation	0.9695		Pearson Correlation	0.847934		
Mean difference hypothesis	0		Mean difference hypothesis	0		
Gl	31		Gl	31		
t-test	-6.53154		t-test	-4.47363		
P(T<=t) one-tailed	1.36E-07		P(T<=t) one-tailed	4.82E-05		
one-tailed critical t	1.695519		one-tailed critical t	1.695519		
p-value	2.73E-07		p-value	9.65E-05		
two-tailed critical t	2.039513		two-tailed critical t	2.039513		

Upper lip height (Right)			Area of both lips		
	Before	After		Before	After
Average	37.75	45.76815	Average	21996.94	30438.69
Variance	352	561,5268	Variance	5.29E+08	1.27E+09
Observations	32	32	Observations	32	32
Pearson Correlation	0.94928		Pearson Correlation	0.976026	
Mean difference hypothesis	0		Mean difference hypothesis	0	
Gl	31		Gl	31	
t-test	-5.4426		t-test	-3.38801	
P(T<=t) one-tailed	3.02E-06		P(T<=t) one-tailed	0.000966	
one-tailed critical t	1.695519		one-tailed critical t	1.695519	
p-value	6.04E-06		p-value	0.001932	
two-tailed critical t	2.039513		two-tailed critical t	2.039513	

The results were further supported by the One-Way ANOVA test, which also demonstrated statistically significant differences between pre- and post-procedure measurements for all four variables (**Table 3**). All p-values were below the 0.05 threshold, confirming that the null

hypothesis of no variation could be rejected. The agreement between the paired t-test and ANOVA results reinforces the reliability of the observed effect, highlighting that lip filling produces consistent dimensional changes across measurement sites.

Table 1. Summary of the results of the one-way ANOVA test for comparison between the variations, before and after the examination, at each measured location.

Measures	F value	P-value	
Upper lip height (left)	426.611	< 0.0001	
Upper lip height (right)	296,219	< 0.0001	
Lower lip height (midline)	200.133	0.0001	
Area of both lips	114,786	0.0019	

Correlation analyses were performed to assess the relationship between lip height variation and age. The graph of lower lip height variation versus age (**Figure 3**) revealed the following correlation coefficients by age group:

20–29 years: approximately 0.55 (moderate positive correlation);

30–39 years: approximately 0.50 (moderate positive correlation);

40–49 years: approximately 0.35 (weak positive correlation);

50–72 years: approximately –0.10 (weak negative correlation).

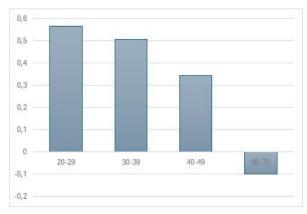


Figure 3. The graph of the variation in lower lip height versus age shows the correlation coefficients for the age groups

This pattern indicates that younger patients exhibit greater vertical gain in lower lip height after filling, suggesting greater tissue responsiveness and dermal elasticity in this age group.

Similarly, when analyzing the correlation between variation in right upper lip height and age (Figure 4), the results showed:

20–29 years: approximately –0.8 (strong negative correlation);

30–39 years: approximately 0.1 (very weak or no correlation);

40–49 years: approximately −0.3 (moderate negative correlation);

60–72 years: approximately 0.4 (moderate positive correlation).

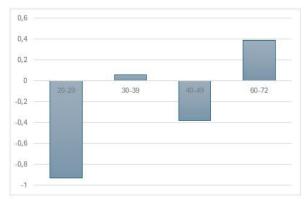


Figure 4. The graph of the correlation between variation in right upper lip height and age across different age groups

These findings indicate that the relationship between age and the upper lip response to filling is more complex and may be influenced by individual factors, such as baseline lip morphology, prior procedures, and regional differences in muscle tone and skin elasticity. The strong negative correlation observed in the youngest group could reflect an inverse relationship between baseline lip fullness and the degree of measurable height increase, as patients with naturally fuller lips may exhibit smaller relative changes after HA injection.

Overall, the quantitative results demonstrate that HA lip filling leads to statistically and clinically significant volumetric enhancement of both upper and lower lips, with variation in response patterns according to age and lip region.

The present study demonstrated, through quantitative pixel-based analysis, that hyaluronic acid (HA) filler effectively increased the vertical height and total area of both upper and lower lips. All measured parameters showed statistically significant post-procedure increases, confirming that HA injection promotes consistent volumetric enhancement and contour definition. From a clinical perspective, these dimensional gains translate into visibly fuller lips with improved definition of the vermilion border and Cupid's

bow — features that are highly correlated with perceived facial attractiveness and youthfulness.

Pixel analysis proved to be a practical and reliable method for documenting outcomes, as it does not require specialized three-dimensional imaging systems or costly equipment. This advantage facilitates broader clinical application and allows for standardized photographic follow-up, even in routine aesthetic practice. Although no previous studies were found that specifically used pixel analysis for lip filler assessment, other investigations have employed twodimensional photographic evaluations or advanced photogrammetry techniques, such as Vectra Quantificare systems [37-43]. These tools have consistently demonstrated that HA lip augmentation improves lip projection, symmetry, and contour, enhancing patient satisfaction and self-esteem [37, Stereophotogrammetric approaches provide high-resolution three-dimensional data that capture subtle soft-tissue changes, thereby improving clinical monitoring and patient communication [42-51]. The findings of the present study suggest that pixel-based analysis may represent a simpler yet effective alternative for documenting measurable changes in clinical and research settings.

In the current investigation, all participants received 1 mL of HA, which aligns with clinical recommendations suggesting 1 mL as the optimal volume for initial lip augmentation, with the option of adding a second session if further volumization is required [43]. The filler used, Restylane Kysse (Galderma), was selected due to its physicochemical properties, including cohesiveness, softness, and low elasticity, which allow for smooth integration into the tissue and natural dynamic movement without rigidity [52]. These attributes make it particularly suitable for the lips, where mobility and expressiveness are crucial. Other studies have also reported favorable results with Restylane Kysse® for vermilion augmentation and contour enhancement [53].

Comparable clinical outcomes have been reported with other HA-based fillers, such as Juvéderm Ultra (Allergan), Belotero Intense (Merz), and Rennova Fill (Rennova), all of which have demonstrated satisfactory patient-reported outcomes and measurable volumetric improvements [15, 54, 55]. However, the degree of correction and aesthetic longevity may vary according to filler rheology, injection depth, and individual patient characteristics.

The correlation analyses performed in this study revealed that younger patients exhibited greater vertical gain in lower lip height after filling, suggesting higher dermal elasticity and tissue responsiveness in this group. This observation aligns with clinical experience: younger skin tends to exhibit higher hyaluronic acid content and better biomechanical integration of fillers. Conversely, the variable correlation patterns observed in upper lip measurements across different age groups likely reflect individual anatomical and functional differences, such as baseline lip thickness,

muscle activity, and prior aesthetic procedures. Clinically, this emphasizes the need for individualized treatment planning, as the same filler volume may yield distinct outcomes depending on the patient's age and lip morphology [56-62].

Conclusion

The results showed a statistically significant change in all upper and lower lip measurements, as well as in the total area, after filling with only 1 mL of HA, as measured by pixel analysis.

In general, the age trend graphs show a decreasing correlation with age (except for the variation in mouth area, which exhibits a strong correlation in the 60-72 age group), indicating possible changes in the pattern of anatomical variation with aging.

Specifically, regarding the variation in lower lip height and age, there is a general trend of decreasing correlation with age. The 20-29 and 30-39 age groups show moderate positive correlations, suggesting a more consistent relationship; in the 40-49 age group, the correlation is weaker; and in the 60-72 age group, the correlation was negative, indicating either an absence of a relationship or an inverse correlation at this stage.

A notable curiosity was that the 20-29 age group frequently exhibited moderate positive correlations across most variables, suggesting that this group may be more susceptible to age-related anatomical changes or may exhibit greater retention/reaction to procedures.

Finally, the mouth area showed the most consistent correlations across age groups (moderate to strong positive), providing robust evidence that HA lip fillers are an effective procedure with a measurable effect on patients' lip characteristics. Longitudinal studies using pixel analysis are suggested to assess the longevity of the procedure.

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